Nature Precedings : doi:10.1038/npre.2009.3637.1 : Posted 17 Aug 2009

The hotspot conversion paradox

April 17, 2002

Nature Precedings : doi:10.1038/npre.2009.3637.1 : Posted 17 Aug 2009

Background

Recombination hotspots (HS) are . . .

- fragile sites where recombination is initiated.
- defined by an activity level, e.g. active vs. inactive allele, and location, e.g. an unique sequence (HSS).

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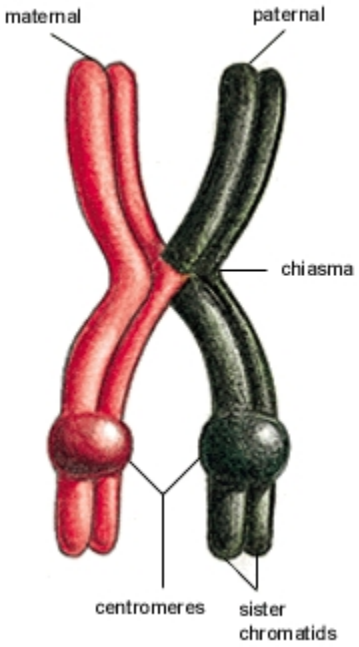
Recombination is important for:

- Genetic reshuffling during sexual reproduction creating new allele combinations within chromosomes and
- ensuring proper segregation of chromosomes.



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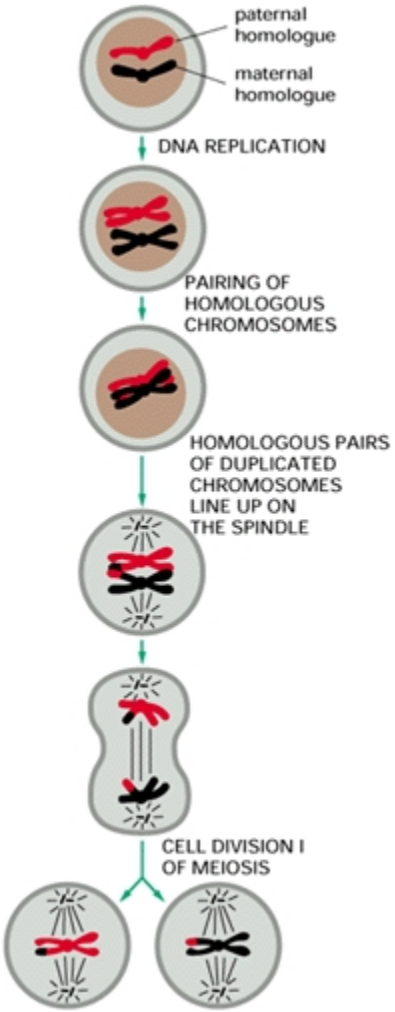

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The hotspot paradox

1. Recombination initiates at an active HS by a double strand break, DSB.
2. The recombinational repair mechanism replace the broken HS with a copy from its homolog chromosome.
3. This predict that if a mutant inactive HS allele in the population it should take over.
4. . . . but functional HS remain ubiquitous!

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A highly incomplete review of HS occurrence

Organism	Identified HS	Comment	Ref
E. coli	χ		Urawa et al. 2001
S. cerevisiae	*HOT1*		Urawa et al. 2001
	ARG4, *HIS4*		Lichten & Goldman 1995
S. pombe	*M26*	ATGACGT	Fox et al. 2000
Hamster		*GNAI3*, 3kb AT rich HSS	Svetlova et al. 2001
H. sapiens		AT rich HSS	Svetlova et al. 2001

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The Hotspot paper"

Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 8058–8063, July 1997
Genetics

The hotspot conversion paradox and the evolution of meiotic recombination

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Communicated by John R. Roth, University of Utah, Salt Lake City, UT, May 30, 1997 (received for review January 13, 1997)

ABSTRACT **Studies of meiotic recombination have re-**
'ealed an evolutionary paradox. Molecular and genetic anal-
has shown that crossing over initiates at specific sites
hotspots, by a recombinational-repair mechanism in
he initiating hotspot is replaced by a copy of its
We have used com or simulations of large popu-
resolution of the repair intermediate fre
crossover between the participating chromc
and related recombinational-repair mech
for both the preferential conversion of
their inactive homologs, seen in fur
gene conversion with rec
organisms (

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The Model

1. Pool of haploid gametes
2. Mutation in + alleles to − alleles, $r^+ \rightarrow r^-$, $a^+ \rightarrow a^-$, ...
3. Gamete fusion
4. Selection
5. Meiosis
6. Pool of haploid gametes
7. ...

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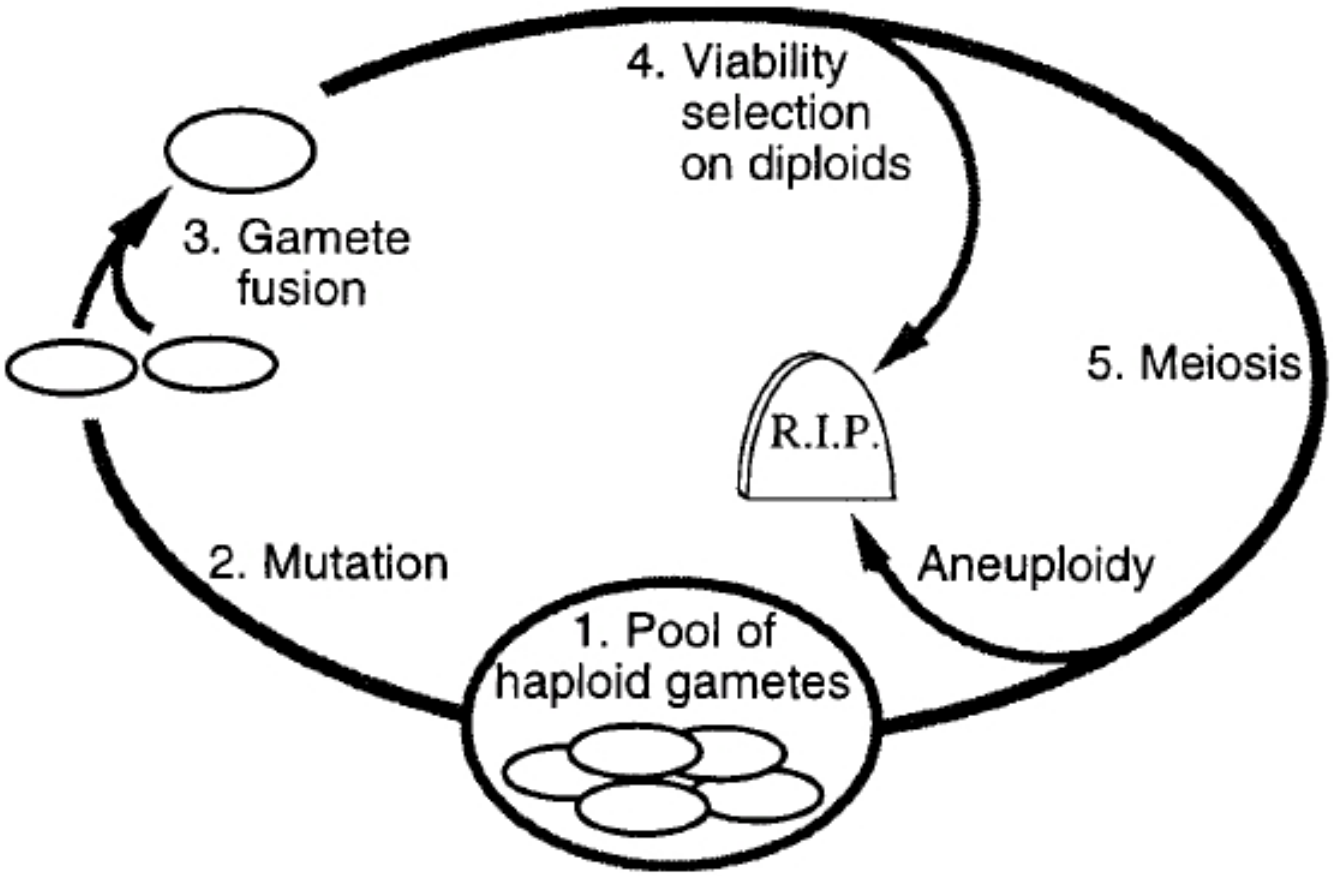
The lifecycle



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1. Pool of haploid gametes

- Each gamete contains one chromosome with one recombination HS, r.
- Initially all gametes carry the active r^+ allele.
- Inactive mutant HS, r^-, arise at frequency $\mu_r = 10^{-8}$ per allele per generation.
- 3 diallelic loci, a^+/a^-, r^+/r^-, and b^+/b^-.
- The a and b loci affect the viability, where the $-$ (minus) alleles are deleterious and $+$ alleles are wild type.
- There are 8 possible haplotypes:
  $\mathcal{H} \in \{[+++], [++-], [+-+], [-++], [-+-], [--+], [---]\}$,
  where $[+++]$ represents $a^+r^+b^+$, ...
- The frequencies of the haplotypes are $z_{[+++]}$, $z_{[++-]}$, ..., $z_{[---]}$.

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How does the frequency of active HS alleles, r^+, change through time?

$$\frac{dz_{r^+}}{dt} = ?$$

where

$$z_{r^+} = z_{[+++]} + z_{[++-]} + z_{[-++]} + z_{[-+-]}$$

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2. Mutation

Let $\mu'_a = (1-\mu_a)$, $\mu'_r = (1-\mu_r)$, and $\mu'_b = (1-\mu_b)$, them the mutation process across generation is a Markov process with a transition matrix $\mathbf{M}$ ($- \rightarrow +$ is not allowed) where the elements M_{ij} is the probability that haplotype i (vertical) mutates to haplotype j (horizontal):

$$\mathbf{M} = \begin{bmatrix}
 & [+++] & [++-] & [+-+] & [-++] & [+--] & [-+-] & [--+] & [---] \\
[+++] & \mu'_a\mu'_r\mu'_b & \mu'_a\mu'_r\mu_b & \mu'_a\mu_r\mu'_b & \mu_a\mu'_r\mu'_b & \mu'_a\mu_r\mu_b & \mu_a\mu'_r\mu_b & \mu_a\mu_r\mu'_b & \mu_a\mu_r\mu_b \\
[++-] & 0 & \mu'_a\mu'_r & 0 & 0 & \mu'_a\mu_r & \mu_a\mu'_r & 0 & \mu_a\mu_r \\
[+-+] & 0 & 0 & \mu'_a\mu'_b & 0 & \mu'_a\mu_b & 0 & \mu_a\mu'_b & \mu_a\mu_b \\
[-++] & 0 & 0 & 0 & \mu'_r\mu'_b & 0 & \mu'_r\mu_b & \mu_r\mu'_b & \mu_r\mu_b \\
[+--] & 0 & 0 & 0 & 0 & \mu'_a & 0 & 0 & \mu_a \\
[-+-] & 0 & 0 & 0 & 0 & 0 & \mu'_r & 0 & \mu_r \\
[--+] & 0 & 0 & 0 & 0 & 0 & 0 & \mu'_b & \mu_b \\
[---] & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1
\end{bmatrix}$$

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3. Gamete fusion

- The mutant haploid gametes fuse at random to produce diploid organisms.
- With 8 possible haplotypes there are 64 possible diplotypes, of which 36 are unique..

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4. Viability selection on diploids

The fitness of a diploid is $w_{ij} = \lambda_r \lambda_{ab}$ where λ_r is a function of the allele at the HS locus:

$$\lambda_r = \begin{cases} 1 & \text{if there are two } r^+ \text{ alleles (homozygous +)} \\ 1 - h_r s_r & \text{if there is exactly one } r^+ \text{ (heterozygous)} \\ 1 - s_r & \text{if there are two } r^- \text{ alleles (homozygous -)} \end{cases}$$

When $h_r = 0$ r^- is recessive, when $h_r = 0.5$ r^- is codominant, and when $h_r = 1$ r^- is dominant. s_r is the selection coefficient ($0 < s_r \leq 1$).

λ_{ab} is a function of the total number of mutant alleles at the a and b loci, thus $\lambda_{ab} \in \{0, 1, 2, 3, 4\}$.

The proportion after selection of each diplotype is thus:

$$\widehat{z} = w_{ij} z_{ij}$$

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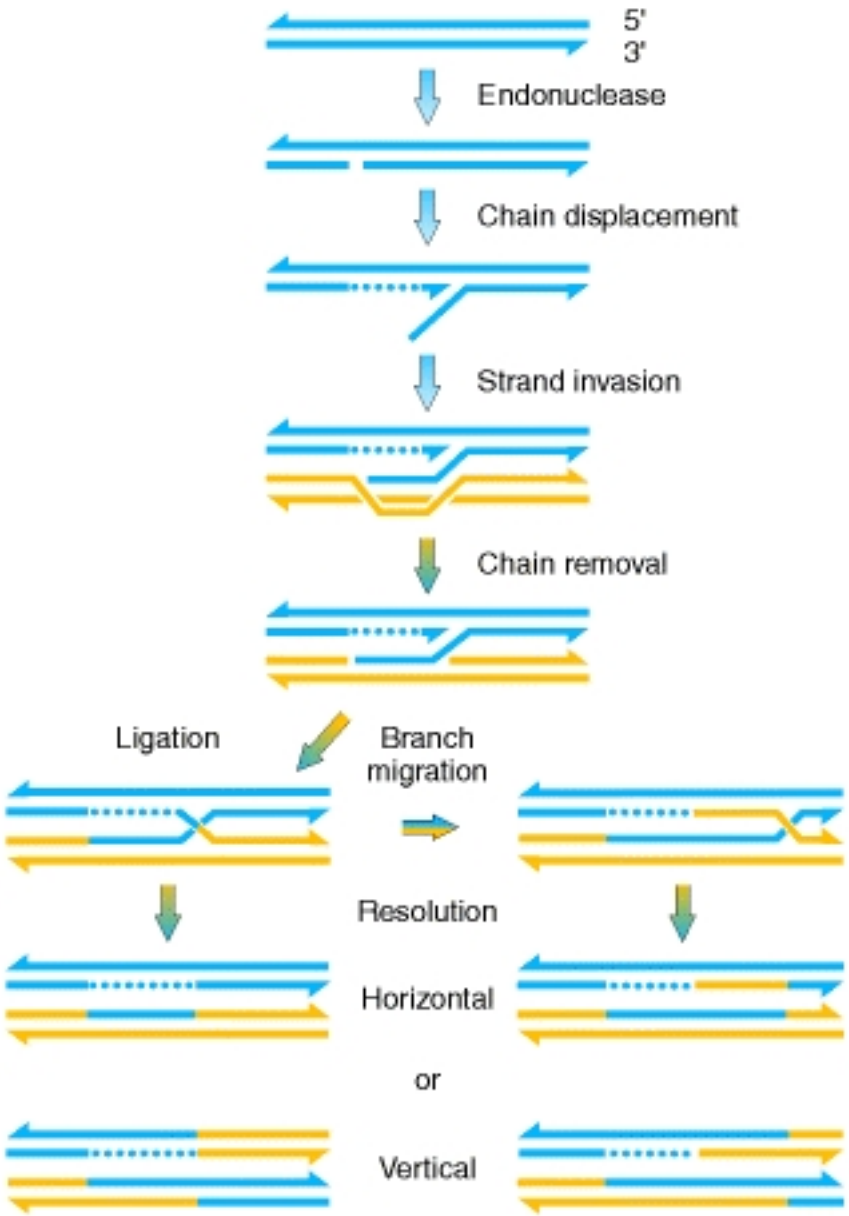

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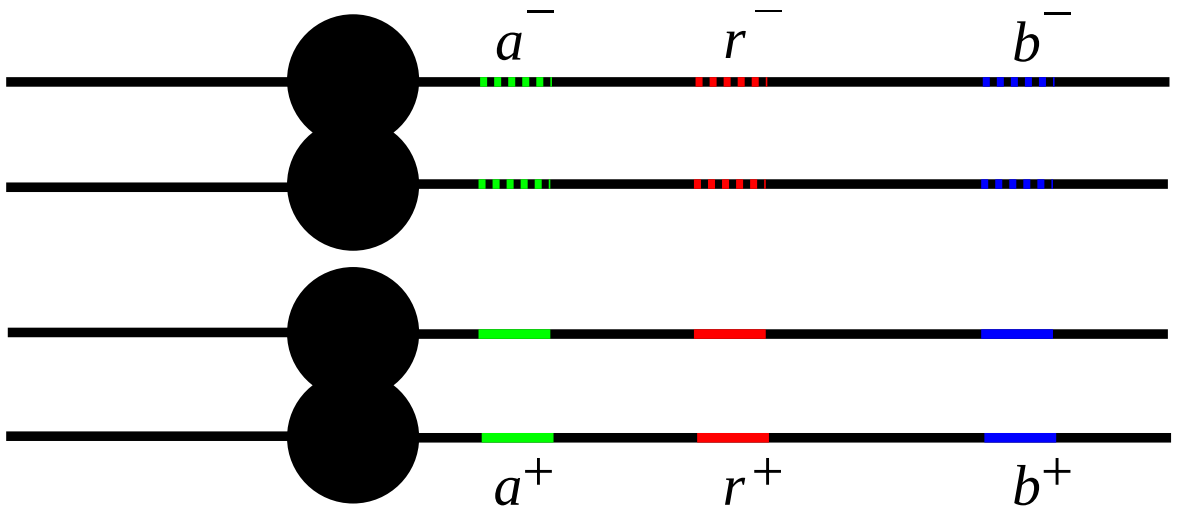
Meiotic recombination 1 — Pairing of homologous chromosomes

Pairing of homologous chromosomes.



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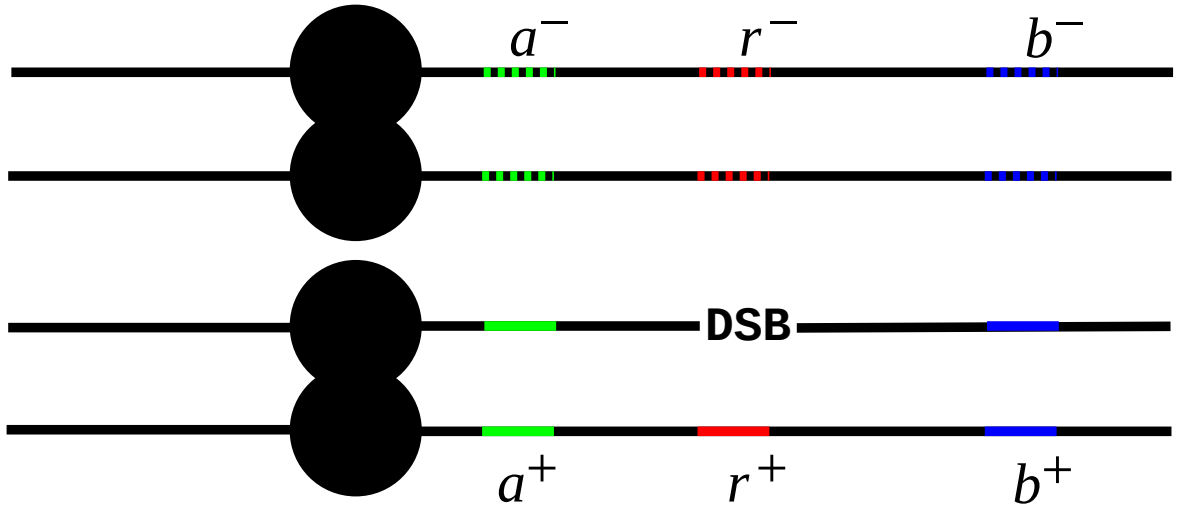
Meiotic recombination 2 — DSB in r^+.

One active HS allele undergoes a double-strand DNA break (DSB).



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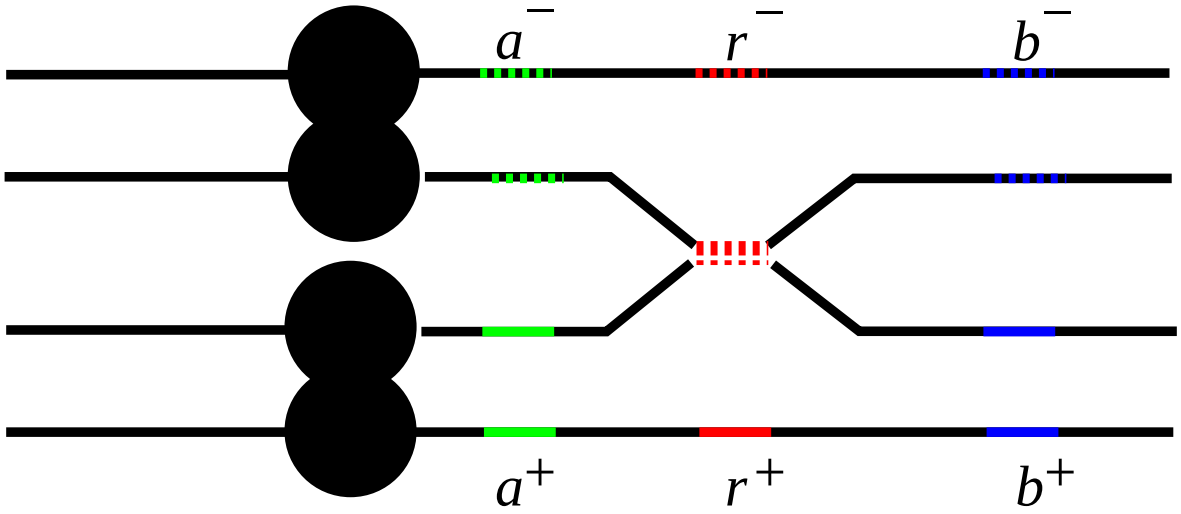
Meiotic recombination 3 — Recombinational repair

Formation of a DNA heteroduplex and the broken chromatid undergoes recombinational repair, using a homologous chromatid as a template.



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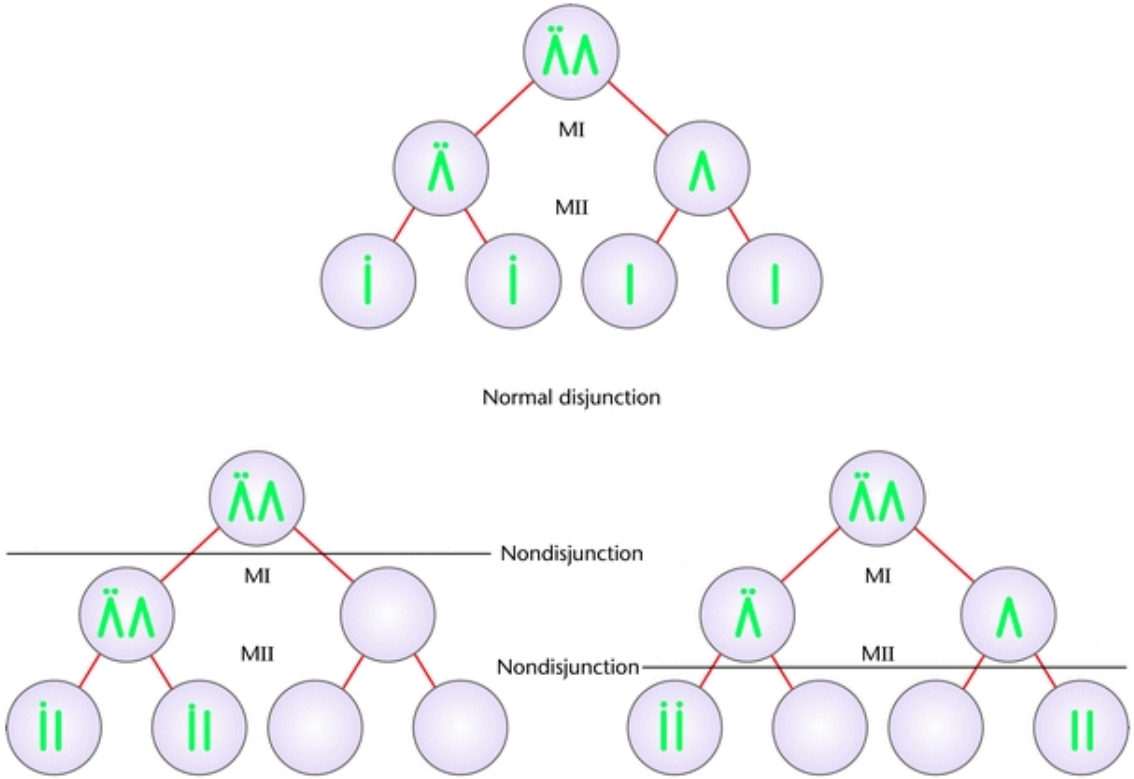
Meiotic recombination 4 — Segregation/disjunction



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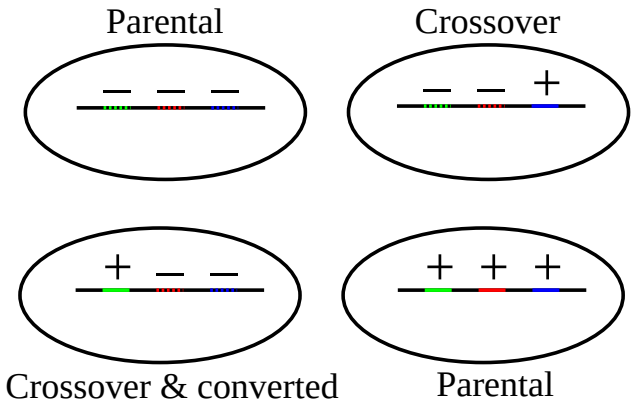
1.



If resolution of the repair intermediate produces a crossover, segregation is accurate and each gamete receives a single chromosome.

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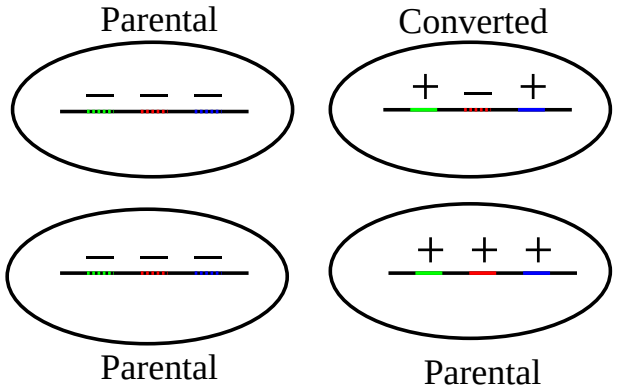
2.



If no crossover has take place, chromosomes may be distributed randomly at meiosis I, giving either four functional gametes ($Pr = 0.5$) . . .

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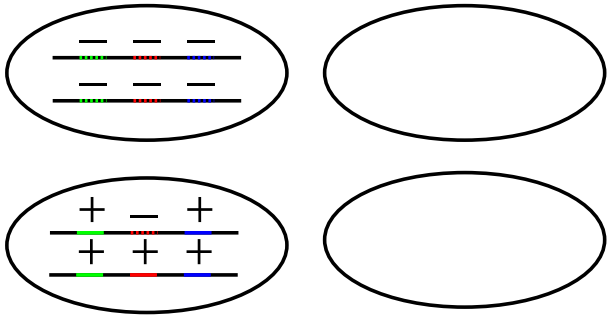
3.



or four aneuploid gametes.

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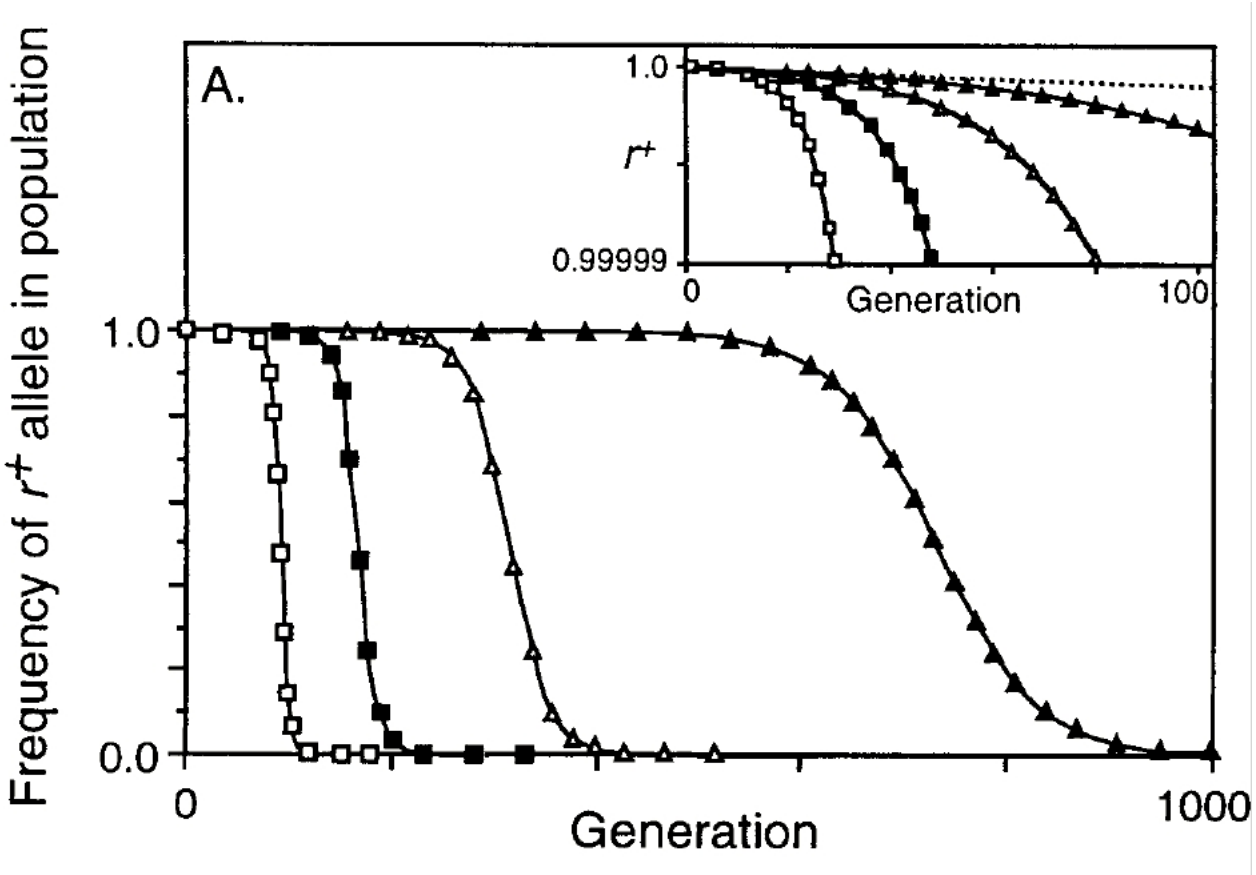
Results — the neutral case

$C \in \{0.2, 0.1, 0.05, 0.02, 0\}$, ($C$: prob. that a r^+ initiates recombination)



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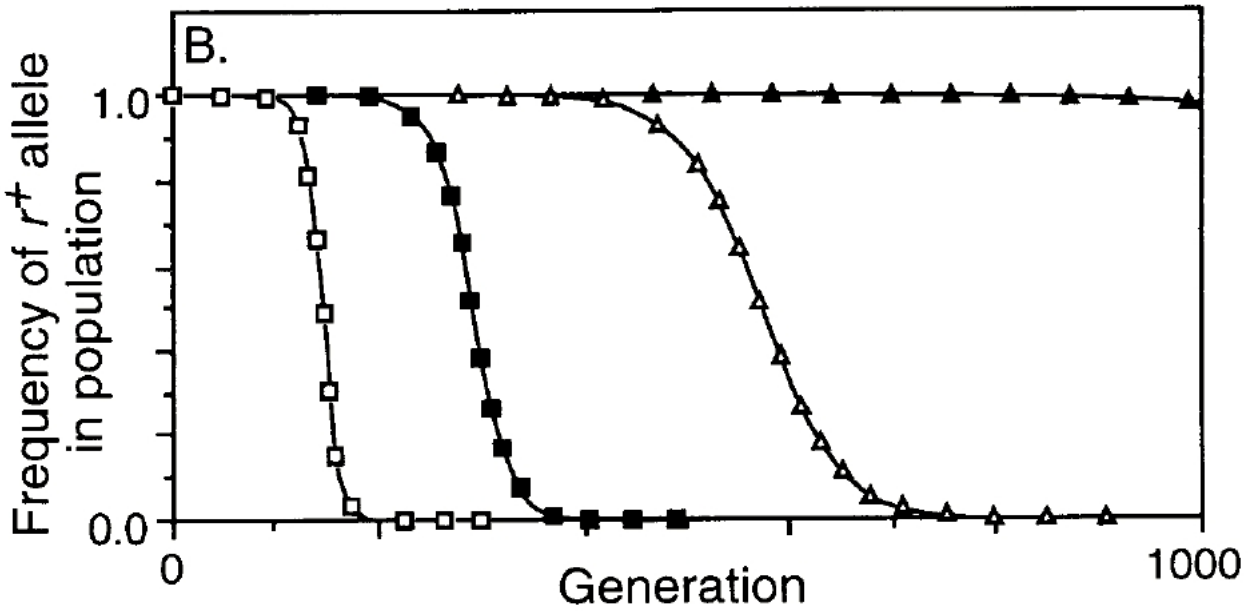
Results — with selection

$C \in \{0.2, 0.1, 0.05, 0.02, 0\}$, ($C$: prob. that a r^+ initiates recombination)



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r^+ is replaced by r^- when . . .

- when there is no selection,
- in obligate sexual populations,
- in facultative sexual populations,
- considering the benefits of accurate segregation and genetic recombination.
- . . . not, however, if an additional, non-meiotic function for hotspots is introduced. Strong selection for this function could allow active hotspots to persist in spite of frequent conversion to inactive alleles.

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The demise of r^+ alleles is inevitable!

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Questions

- Are HS relative or absolute?
  - Relative — DSB frequency is relative to other sites at the chromosome. The HS is not defined in terms of as sequence (HSS) but rather by exogen factors such as chromatin structure and interactions with other chromosomal elements (Lichten & Goldman 1995).
  - Absolute — HS are defined in terms of an specific and unique sequence, HSS, e.g. the octameric χ sequence GCTGGTGG in *E. coli*
- Is there a correlation among various types of fragile sites, e.g. mutational hotspots . . .

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Approaches!

"*If the facts don't fit the theory, change the facts.*"

–Albert Einstein

- Analyse previous model, improve and extend, e.g. multiple HS loci, fluctuating selection, gradients of HS activity, . . .
- Details of the molecular mechanism of recombination. ". . . the nature of recombination prone regions remains obscure." (Svetlova et al. 2001).

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Ideas!

Pre-recombination mechanisms

- r^+do not actually commit suicide? What is the molecular mechanims of the DSB?
- HS activity not binary (on/off) but rather a continuum.
- HS may be "insensitive" to base pair mutations, it may be more relevant to use per base pair mutation rate.

Post-recombination mechanisms

- Regeneration of r^+ alleles through mutations, i.e. ${r_1}^+ \rightarrow r^- \rightarrow {r_2}^+$ where ${r_1}^+ \neq {r_2}^+$.
- Several HSS may result in activity, e.g. *TGACGT(A/C) in *S. pombe* (Fox et al. 2000). The activity level may vary depending on the HSS.